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Review

Defining Valid Chronic Stress Models for Depression With Female Rodents

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ABSTRACT

Women are twice as likely to experience depression than men, yet until recently, preclinical studies in rodents have focused almost exclusively on males. As interest in sex differences and sex-specific mechanisms of stress susceptibility increases, chronic stress models for inducing depression-relevant behavioral and physiological changes in male rodents are being applied to females, and several new models have emerged to include both males and females, yet not all models have been systematically validated in females. An increasing number of researchers seek to include female rodents in their experimental designs, asking the question "what is the ideal chronic stress model for depression in females?" We review criteria for assessing female model validity in light of key research questions and the fundamental distinction between studying sex differences and studying both sexes. In overviewing current models, we explore challenges inherent to establishing an ideal female chronic stress model, with particular emphasis on the need for standardization and adoption of validated behavioral tests sensitive to stress effects in females. Taken together, these considerations will empower female chronic stress models to provide a better understanding of stress susceptibility and allow the development of efficient sex-specific treatments.

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Depression affects twice as many women as men (1–4), differing qualitatively by sex, with women reporting greater severity and more atypical and somatic symptoms (5–7). Whereas in men, depression is often comorbid with externalizing and substance use disorders, in women, depression is more often comorbid with internalizing and anxiety-related disorders (5). Sex also affects treatment efficacy (8,9). Understanding mechanisms of sex and gender differences is therefore essential to understanding depression and developing widely effective treatments.

Despite clinical realities, the majority of animal studies include only males, reflecting a broader issue in neuroscience (10). In chronic stress and depression literature, male rodent studies continue to far outnumber female studies (Figure 1). This may be attributed to the erroneous dogma of female behavior being too variable, affected by hormones, and discrepant relative to an assumed male norm (10–12). However, variability in cycling females is not greater than in males (13). Testosterone in dominant males can be five times higher than in subordinate cagemates (14), and circadian fluctuations interact with stress and animal strain (15,16). Recognizing the urgent need to address sex bias in preclinical research, various funding agencies issued recommendations for including both sexes (12,17), spurring a rapid increase in research in females. As with any rapid change, this has also raised challenges for the field.

Male bias in neuroscience continues to shape experimental design in less visible ways. With few exceptions, rodent stress models were designed and optimized in males and only later used in females (12,18). Yet, paradigms established in males may not optimally capture stress effects in females. Most ethological stress models exploit male-specific behaviors, and ethological validity may not extend to females. Behavioral tests developed in males are used in females with an untested assumption of behavioral similarity. Females and males may be vulnerable to different types of stressors (e.g., social vs. aggression), and current behavioral tests may be less suited to females (11,12,18). We overview considerations for rigorous research in females to understand stress susceptibility and depression-like behavior in both sexes, set out criteria for defining good female stress models, and review current stress models amenable to adult female rodents.

WHAT MAKES FOR A GOOD FEMALE STRESS MODEL?

Rigorous animal models address several key criteria: 1) face validity: analogy of the model behavioral phenotype and clinical symptoms, 2) construct validity: similarity of neurobiological mechanisms and etiology for modeled phenotypes and disorder, and 3) predictive validity: amelioration with clinically effective, but not ineffective, treatments (19–21). External validity—ease of reproducibility across laboratories—is also critical (22); complex models are challenging to replicate. Most female stress models build on long-established male models, yet reevaluating validity in females is important because males and females are different. In adapting models to females, essential qualities may be lost, particularly ethological validity and appropriateness of behavioral outcomes.

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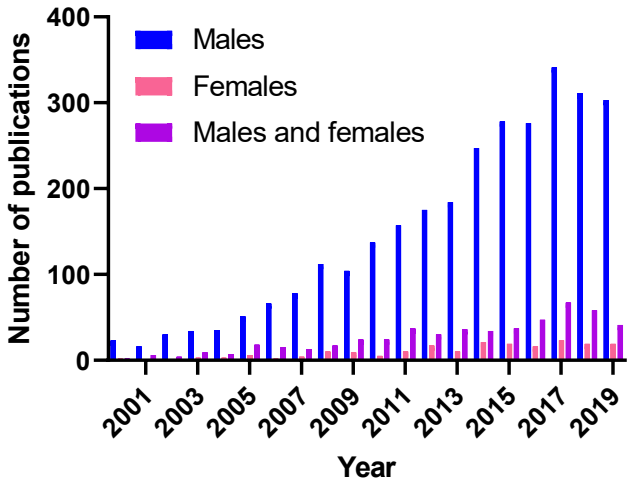

Figure 1. Histograms representing the number of publications per year using male rodents only, female rodents only, or both sexes for studies on chronic stress and depression between the years 2000 and 2019. PubMed advanced searches: "Chronic stress AND depression AND rodents," "Chronic stress AND depression AND female rodents NOT male rodents," "Chronic stress AND depression AND male rodents NOT female rodents."

Ethological Validity

Sexual dimorphisms (Supplement) in physiology and behavior necessitate evaluating ethological validity in each sex. Evolution has shaped sex-specific behavioral adaptations (11): male rodents are territorial, prone to same-sex aggression; females are more social, exhibiting less pronounced dominance hierarchies in nonreproductive states (11,12,23). Females may be sensitive to impoverished social environments, e.g., isolation, and males to conspecific aggression. Fitting females into already validated male models rather than developing female-specific ethologically valid models risks further entrenching male bias.

Female-Adapted Behavioral Outcomes

Female rodent behavior can differ in standard tests, requiring careful interpretation (12,24). Relative to males, females explore an open field more, spend more time in open arms of an elevated plus maze, and are more active during forced swim tests, phenotypes conventionally interpreted as reduced anxiety- and depression-like behavior (see Definitions of 'depression-like' and 'anxiety-like' behavior in the Supplement) (25–31). Yet, increased anxiety and mood disorders in women suggest a discrepancy between clinical and preclinical research, highlighting the need to reconsider behavioral measures. In some cases, a methodological adjustment could suffice; for example, longer sucrose preference tests (24 hours) reveal impairments in females, potentially because of different consumption patterns (32–34), and ultrasonic vocalizations can help detect chronic mild stress effects (35).

Female rodents may also exhibit distinct behaviors not captured by standard measures, invalidating conclusions from single behavioral metrics; fear-conditioned female rats freeze less than males yet demonstrate learning in rearing and darting (24,36). Expanded characterization in common tests can help capture female behavior; in social interaction, scoring social vigilance, in addition to social approach, reveals high levels of this behavior in females (37). Novel, naturalistic assessments could also be considered; stressed females build less developed nests than nonstressed control females (38). Data-driven analyses may help rigorously characterize stress-sensitive female-specific behavior. Rigorous female stress models require a range of measures informed by female-specific behavior.

Model Reliability/Consistency

Models must be replicable within and across laboratories. Inconsistencies may arise from varied implementation and testing. For example, chronic mild stress, consisting of daily varying stressors applied unpredictably, is challenging to consistently implement given the myriad of stressors (39). Standardization is more easily achieved with simple models. Behavioral protocols also affect replicability. For example, in the sucrose preference test, sucrose concentration, duration, food/water restriction, sucrose preexposure, water source, time of day, variables measured, and single housing prior to testing all can alter results (39–41).

Animal husbandry, rodent strain, and supplier also introduce variability (see Supplement) (11,39,42). Some experimenters track estrous cycle with daily vaginal cytology, yet many do not. While habituation reduces the stress inherent to manipulations, this is an important source of variability, and it is debated whether tracking estrous is necessary or appropriate (12).

Availability of an Equivalent Male Model

Inferring male-female differences in susceptibility and resilience from differing stress manipulations is problematic, necessitating similar models in males and females for some questions (discussed below).

CHRONIC STRESS MODELS CURRENTLY USED IN ADULT FEMALES

Learned Helplessness

Learned helplessness, failure to escape an escapable aversive stimulus after previous inescapable stress (43), is one of the longest-established stress models (43–46). Sex differences in escape complicate interpretation, and reported variable efficacy urges caution in using this model in females (see Supplement).

Chronic Corticosterone

To eliminate variability of chronic stress, researchers have attempted to pharmacologically mimic stress with exogenous corticosterone (CORT) (47). This model is not optimal for use in females. Endogenous CORT levels vary across the day in interaction with sex (47), CORT does not fully recapitulate stress-induced hormonal signaling, and the importance of CORT to depressive-like behavior in females has been questioned (48) (see Supplement).

Chronic Restraint Stress

This model induces anxiety-like behaviors in both mice and rats (49–51), with variable effects on depression-like behavior (52,53). While easy to implement (see Supplement), models incorporating varied stressors are preferable.

Chronic Mild Stress

Chronic mild stress (CMS), or chronic mild unpredictable stress, is widely used to study depressive-like behavior (39,42,54). Over several weeks, unpredictable mild stressors are administered, including changes in husbandry, social environment, and food or water deprivation. In male rodents, CMS induces dysregulated hypothalamic-pituitary-adrenal axis activity and anxiety-like and anhedonic behavior reversible by chronic, but not acute, antidepressants (55–57). While CMS has been useful in males, it is demanding in time and space, and its reliability is challenged by inconsistent findings (20,39,58). This may relate to animal husbandry and strain differences (see above), but likely also to the inherently variable nature of the stress. Publications rarely fully detail the precise stressor schedule and timing, often simply listing stressors applied once or twice daily across a (variable) period of weeks, sometimes not detailing stressor application (e.g., white noise without specifying dB or duration) or how variably stressors are applied (e.g., cage tilt for 1–24 hours). While this variability is optimal in maximizing unpredictability, it is problematic in obstructing precise replication.

Nevertheless, CMS is as easily applied in development or adulthood to females as males, allowing direct investigation of sex differences (42) to reveal greater female stress susceptibility (32,59). Fluoxetine prevented depressive-like behaviors, indicating good predictive validity of CMS in female rats (60). CMS results in mice are variable: CMS in C57BL/6 females increased anxiety- and depressive-like behaviors, as well as CORT in some studies (52,61); others report only altered social behavior and response to novelty in female C57BL/6 and CD1 mice (23,62). Adopting detailed standardized procedures, a generic strain and a wider array of validated behavioral tests could increase utility and validity of this model in females as a rigorous model for probing sex differences in stress susceptibility.

Chronic and Subchronic Variable Stress

Chronic variable stress (CVS) is similar to CMS in that animals are exposed to repeated stressors across a period of weeks, yet the schedule of stressors is much less variable and, on average, more intense. Mice are exposed to 1-hour daily stressors alternating among inescapable shocks, tail suspension, and tube restraint, generally for 21 or 28 days (63–65). Simplicity and standardization could facilitate replication. Similar to CMS, CVS can be used in males and females (63–65), with depressive-like behaviors in both sexes persisting 30 days after CVS (64). CVS has been particularly useful in identifying distinct transcriptional mechanisms underlying similar behavioral phenotypes, i.e., convergence (65). CVS effects have not been reported in females in forced swim tests (64,65), emphasizing the importance of a range of behavioral tests.

Subchronic variable stress (SCVS), an abbreviated 6-day CVS that induces depressive-like behavior in females but not males (64,66,67), has been applied in C57BL/6 mice to investigate mechanisms of sex differences in susceptibility (67–71). CVS duration can be titrated to probe degrees of susceptibility in each sex. We used 4-day SCVS in females and 21-day CVS in males to induce group-level differences with within-group variability to probe neural mechanisms of behavioral susceptibility (72). Subthreshold SCVS, typically 3 days for females and 6 days for males (65), which does not induce depressive-like behaviors in control mice, can probe susceptibility mechanisms, e.g., gene manipulations. Overall, CVS is a standardized model easily used in both sexes and adaptable to reveal sex differences in susceptibility, recapitulating the greater depression vulnerability in women. Reproducing CVS across more laboratories is important to confirm reliability and utility for studying both sex differences and female-specific mechanisms.

Social Isolation

Social isolation is a recognized risk factor for depression, providing an ethologically relevant rodent stress model (73,74). Extended individual housing of mice or rats can be studied across development (53,74). Ethological relevance of social isolation may vary with sex given differing male and female same-sex group dynamics, with more aggression among males (11). In mice, 1-week single housing increases anxiety-like behavior in females, but not males, and short-term single housing is the least stressful condition for males (75,76). Extended social isolation in adult males induces anxiety- and depressive-like behaviors (74,77). Social isolation induces depressive- and anxiety-like behaviors in adult female prairie voles (78), C57BL/6 mice (79,80), and Sprague Dawley rats (81), effects reversed by chronic fluoxetine or acute ketamine. Overall, pre- or postweaning social isolation is effective for studying enduring early-life stress effects in females and sex differences in susceptibility. Although duration is a consideration, beyond rehousing, little work is required, and this model accommodates developmental stress in both sexes. Systematically determining minimum isolation duration for susceptibility, as well as phenotype durability, would facilitate standardization and wider use.

Social Instability Stress

Social instability stress (SIS) alternates periods of crowding and isolation over several weeks, rotating cagemates for an unstable social environment wherein mice must repeatedly reestablish hierarchies (82–85). SIS induces physiological and behavioral alterations in males and females during adolescence or adulthood (83,86,87). In female adult rats, SIS altered CORT and depressive- and anxiety-like behaviors, although findings vary (82,85,88–90). Differing strains or protocol length could explain inconsistent physiological changes across studies. Few studies have used SIS in adult mice, and while anxiety- and depressive-like behaviors are observed, CORT varies (23,83). A meta-analysis of 17 SIS rodent studies reported some conflicting findings (84), likely because of methodological inconsistencies, including animals per cage, cagemate change frequency, duration, male presence or

absence, or species and strain (22,84,91). Establishing a standardized protocol in common strains would increase utility of this ethologically relevant stress model in adult females, already a powerful tool for chronic stress in juveniles.

Social Defeat Variants

Chronic social defeat stress (CSDS), one of the most common stress models in male rodents, exposes an intruder mouse to brief aggressive encounters in the home cage of a larger aggressive mouse, e.g., CD1 retired breeder, daily for 10 days (92–95). Between daily defeats with novel aggressors, the intruder mouse remains housed adjacent to the aggressor across a perforated plexiglass divider allowing sensory contact. After defeat, about two thirds of C57BL/6 mice show susceptibility, with increased anxiety- and depressive-like behaviors, including reduced open field center time, social avoidance, and decreased sucrose preference. Resilient mice show increased anxiety-like behavior but unaltered social behavior or sucrose preference, enabling investigation of both susceptibility and resilience. Chronic antidepressants (e.g., fluoxetine, imipramine) or acute ketamine reverse susceptibility (96,97). Exploiting male rodent territorial aggression, this model has strong ethological relevance, yet standard CSDS does not work in female mice; most female rodents are not routinely aggressive, and aggressive male conspecifics generally do not attack females.

Several adaptations have emerged for female CSDS, employing manipulations to induce male-female or female-female aggression: applying male CD1 urine onto female C57BL/6 mice (98), lesioning mediobasal-hypothalamus in female Wistar rats to induce female-female aggression (85), and chemogenetically activating ventrolateral-ventromedial-hypothalamus ERα-expressing neurons in male transgenics to induce pathological aggression (99,100). While these models increase anxiety- and depressive-like behaviors and/or CORT, there are caveats. Manipulations required to induce female-directed aggression present logistical challenges and, consequently, have not been widely adopted. In the recently developed chronic nondiscriminatory social defeat stress model, a male and female C57BL/6 intruder dyad is simultaneously defeated (101), overcoming practical challenges. Although females were attacked less frequently, susceptible and resilient phenotypes emerged in both sexes. With the possible exception of chronic nondiscriminatory social defeat stress, female-targeted CSDS adaptations artificially induce a behavior not normally observed, and aggressive encounters may not be highly relevant stressors for female mice. Although not necessarily a fatal flaw—various stress models use artificial stressors—a widely appreciated strength of CSDS in male mice is its ethological relevance, yet this strength does not extend to female-adapted models. An ideal female model would exploit the behavioral repertoire of females, rather than induce a male-specific behavior in females.

Female-female aggression occurs in specific circumstances; lactating females will defend their pups and aggress intruders (102). Exposing female Wistar rats to lactating Long Evans rats for six daily defeats induced depressive-like, but not anxiety-like, behaviors or altered CORT (103). A 21-day protocol in female Long Evans rats increased CORT, disrupted estrous cycle, decreased weight gain, and reduced saccharine preference (104). Similar manipulations have been attempted in mice; in CD1 females, a single defeat with a lactating dam increased CORT and anxiety-like behaviors (105), but this has not been replicated. Strains or species that naturally show female-female aggression, including Syrian hamsters and California mice (102,106,107), enable direct comparison of males and females, ideal for probing differential susceptibility. Socially defeated female, but not male, California mice show reduced social interaction and reactive coping, persisting up to 10 weeks (106,108) and reversible by chronic, but not acute, antidepressants (109). California mice are a promising model for sex-specific mechanisms of stress susceptibility, although scarcity of genetic tools and difficulties obtaining mice have limited wider adoption.

A recently developed female-female aggression model uses female Swiss Webster (Carworth Farm Webster [CFW]) mice, housed with castrated CFW males, to aggress C57BL/6 females (38,110). A 10-day defeat increased CORT and activated similar brain regions in socially defeated males and females. Defeated females avoided an unfamiliar C57BL/6 female in their home cage, and ketamine reversed this. In a novel arena social interaction test, females showed hypervigilance but neither social avoidance nor anxiety-like behavior in a light/dark box. Defeated females also explored a novel object less and built incomplete nests. This novel model is particularly interesting in using an ethological stressor in a common mouse strain and in extending behavioral assessment to consider additional female-relevant behaviors, including home cage social interaction and nest building. Stable susceptibility was only induced in 12-week-old females, possibly because of estrous cycle irregularities in younger mice, a key consideration for this model. While the logistics of the large number of mice (female and castrated male CFW) are a potential barrier, if firmly established, this could be a powerful model to study both female-specific mechanisms and sex differences in susceptibility in parallel with males in a standard social defeat paradigm.

Witness defeat, or vicarious defeat, induces social stress circumventing physical aggression. A mouse or rat, placed across a perforated partition, witnesses an intruder being physically defeated. Witness defeat effectively isolates emotional and psychological components of stress, an important factor in major depressive disorder and other stress-related disorders, indistinguishable from physical stress in standard CSDS (111–113). Witness defeat increases CORT and anxiety- and depressive-like behaviors in male mice and rats, although often with modest effect sizes (111–115), with some effects persisting, or increasing, 30 days after stress. Similarly applied in females, witness defeat in C57BL/6 females increased CORT, decreased social interaction and sucrose preference, and increased immobility in a tail suspension test (116), with social interaction deficits reversed by acute ketamine or chlordiazepoxide, a benzodiazepine. In female Sprague Dawley rats, witness defeat increased depressive- and anxiety-like behaviors, CORT, and proinflammatory cytokines (117). While witness defeat has good validity in females, suitable for probing both sex differences and sex-specific effects of emotional stress, male witness defeat produces smaller effect sizes between defeated and control animals than

Table 1. Summary of Female Stress Models Noting Stressor and Major Strengths and Weaknesses

Stress Model	Source of Stress	Strengths	Limitations	Key References
LH	Inescapable shock	Easy to implement Can be used in parallel in males	Less escape failure in females potentially because of differences in activity or shock sensitivity Short-lasting effects	Dalla *et al.*, 2008 (121) Caldarone *et al.*, 2000 (122)
CORT Administration	Exogenous CORT	Decreased variability in behavior Easy to implement Can be used in parallel in males	Complicated by sex differences in HPA axis activity Poor relationship between dosing and circulating levels	Kott *et al.*, 2016 [(47), rats] Kokras *et al.*, 2021 [(48) for review]
CRS	Physical restraint	Easy to implement Can be used in parallel in males	Habituation to stress over time	Ngoupaye *et al.*, 2018 (123)
Social Isolation	Social isolation	Juvenile (early life, adolescent) or adult stress model Naturalistic stressor Can be used in parallel in females	Long procedure Variability in protocols	Martin and Brown, 2010 [(79), mice] Sarkar and Kabbaj, 2016 [(81), rats]
SIS	Alternating phases of crowding and isolation; unstable social group	Juvenile (early life, adolescent) or adult stress model Naturalistic stressor Can be used in parallel in males	Long procedure Variability in protocols	Goñi-Balentziaga *et al.*, 2018 [(84) for review]
CMS	Repeated, varied mild unpredictable stressors over several weeks	Juvenile (early life) or adult stress model Translational to human daily stress Can be used in parallel in males	Long procedure, labor intensive, and requires lots of space Variability in protocols	Franceschelli *et al.*, 2014 [(42) for review]
CVS	Repeated daily alternating 1-hour stress (inescapable foot-shock, tail suspension, tube restraint) applied over several weeks	Standardized protocol Can be used in parallel in males	Nonethological stressors Long procedure	Labonté *et al.*, 2017 (65)
SCVS	The same daily alternating stressors as CVS applied across a shorter duration	Mimics greater susceptibility in women Protocol length can be titrated for specific questions Can be used in parallel in males	Nonethological stressors	Hodes *et al.*, 2015 (67)
CSDS With Male Urine	Aggressive encounter with male mouse	Can be used in parallel to standard CSDS in males	Labor intensive Male-female aggression not ethological	Harris *et al.*, 2018 (98)
CSDS With Surgical Manipulations to Induce Aggression	Aggressive encounter with male mouse	Can be used in parallel to standard CSDS in males	Labor intensive Male-female aggression not ethological	Takahashi *et al.*, 2017 [(99), mice] Haller *et al.*, 1999 [(85), rats]
CNSDS	Aggressive encounter with male mouse and a simultaneously aggressed male	Females and males tested simultaneously	New model Male-female aggression not ethological	Yohn *et al.*, 2019 (101)
CSDS With Lactating Female	Aggressive encounter with lactating female rodent	Can be used in parallel to standard CSDS in males Naturalistic stressor	Requires breeding or purchasing of lactating females	Bourke and Neigh, 2012 [(103), rats] Jacobson-Pick *et al.*, 2013 [(105), mice]
CSDS With Female California Mice	Aggressive encounter with female mouse	Can be used in parallel to standard CSDS in males Naturalistic stressor	California mice are not widely available; limited genetic tools	Trainor *et al.*, 2011 (106)

Table 1. Continued

Stress Model	Source of Stress	Strengths	Limitations	Key References
CSDS With Swiss Webster Resident Mice	Aggressive encounter with female mouse	Can be used in parallel to standard CSDS in males Naturalistic stressor	Large number of animals required New model	Logan *et al.*, 2019 (110)
Witness Defeat	Witnessing aggressive encounter of 2 male rodents	Males and females tested simultaneously with CSDS or in parallel with both sexes used as witnesses No physical injuries Psychological/emotional stress	New model Effects not as strong as CSDS	Iñiguez *et al.*, 2018 [(116), mice] Finnell *et al.*, 2018 [(117), rats]

CMS, chronic mild stress; CNSDS, chronic nondiscriminatory social defeat stress; CORT, corticosterone; CRS, chronic restraint stress; CSDS, chronic social defeat stress; CVS, chronic variable stress; HPA, hypothalamic-pituitary-adrenal; LH, learned helplessness; SCVS, subchronic variable stress; SIS, social instability stress.

physical defeat (111,114), and females may be more susceptible, leading some researchers to compare female witnesses with physically defeated males. Although a seemingly reasonable strategy, reported outcome differences between physical and emotional stress urge caution, given that this approach confounds sex differences and stressor type (physical vs. emotional) (114). Few studies have used this model in adult females, suggesting potential difficulties establishing this model across laboratories. Witness defeat effects are smaller than CSDS and potentially more sensitive to varying conditions such as housing or baseline anxiety levels. Wider replication across groups could help establish witness defeat as a rigorous and reliable female stress model.

SEX DIFFERENCES OR DIFFERENT SEXES?

An important distinction exists between studying animals of different sexes and studying sex differences. The former acknowledges the reality that different sexes exist, are different, and should be studied in most research. The latter defines a field of research devoted to characterizing and understanding biological mechanisms defining differences between sexes. To understand biological mechanisms of depressive-like behaviors, two fundamental questions need to be addressed: what is the biological basis for sex differences in stress susceptibility, and what are the biological mechanisms of stress susceptibility in each sex? Each question necessitates distinct considerations for experimental design.

What Is the Biological Basis for Sex Differences in Stress Susceptibility?

Determining the biological basis of sex differences in stress susceptibility requires stress models, uniformly applied to both sexes, to reveal differences in behavioral susceptibility that can be probed for underlying mechanisms. In a recent example of this approach, Williams *et al.* (71) investigated the role of gonadal hormones on ventral hippocampus–nucleus accumbens (vHip-NAc) pathway activity in increased female stress susceptibility. A 6-day CVS protocol known to induce susceptibility in females, but not males, reduced sucrose preference in CVS-exposed females paralleled by increased excitability of vHip-NAc neurons. Orchiectomized CVS-exposed males showed a female-like decreased sucrose preference and increased vHip-NAc excitability, and testosterone-treated females showed male-like resilience to CVS. By probing differential behavioral adaptation in the same stress model, this study established how sex hormone–dependent differences in excitability within a specific brain circuit can drive sex differences in susceptibility.

What Are the Biological Mechanisms of Stress Susceptibility in Each Sex?

Investigating mechanisms of stress susceptibility in each sex requires models that induce susceptibility in both sexes; different stress procedures may be required. For example, to determine the role of the vHip-NAc pathway in stress susceptibility in male and female mice, we recently used sex-specific CVS protocols varying in duration (females, 4 days; males, 21 days) specifically to induce similar levels of depression- and anxiety-like behaviors (i.e., decreased social

interaction, increased passive coping, and decreased open field center time) in each sex (72). In vivo fiber photometry demonstrated that prestress vHip-NAc neural activity predicted degree of stress-induced changes in anxiety-like and social interaction behaviors in both sexes and that stress increased vHip-NAc activity in both sexes, although with sex-dependent effects on neural activity during social interaction. By applying different stress protocols to induce similar behavioral phenotypes, these experiments established that vHip-NAc neural activity is predictive of future stress susceptibility in both males and females and identified a common synaptic mechanism of stress susceptibility.

Juxtaposing these studies exemplifies the critical role of experimental design in identifying and understanding sex differences, sex-specific and shared susceptibility mechanisms, and how diverse approaches reveal complex interactions of sex in mechanism. Sex differences may be present at any level; similar behavioral phenotype may not indicate common underlying mechanisms. Although beyond the present scope, a further critical question should be acknowledged: what are the mechanisms of susceptibility to sex-specific phenomena, such as perinatal depression and anxiety? Here, experimental designs focused exclusively on females are appropriate. By clarifying the question and designing experiments with rigorous fit-for-purpose models, research employing complementary approaches can inform our understanding of male and female biology, building a solid foundation for effective, sex-specific treatment.

THE IDEAL MODEL: CONCLUSIONS AND FUTURE DIRECTIONS

While neuroscience has long overlooked female rodents, the scientific community is beginning to recognize the importance of investigating both sexes, taking efforts to develop or adapt existing stress models. Several established stress paradigms exist for juvenile and adult females, and new ones continue to emerge. There is a pressing need for standardization, informed by detailed parametric studies, to support replication across laboratories. While most models can be easily implemented in both sexes, certain logistical limitations (Table 1) may increase risk of replication failure, posing particular challenges for those new to female research less familiar with procedures. Social stressor models are ideal for probing female-specific susceptibility given increased female sensitivity to social stress. Adopting a standardized battery of female behavioral tests would support comparisons across laboratories to determine model rigor with choice of behavioral tests informed by sex differences. While locomotor and anxiety tests such as the open field can be included, assessing social behavior is strongly recommended, and other behaviors such as nest building, an indicator of animal welfare (118,119), could bring value. Ultimately, further research is needed to define female-specific behavioral adaptations to chronic stress to move beyond existing notions of stress behavior defined in males. Finally, antidepressant efficacy in females has not been tested in some common models, suggesting an assumption of male-female similarity, and the need for more research to assess predictive validity of female models. Indeed, female rats seem to show greater sensitivity to ketamine, even at a dose ineffective in males, a difference likely due to gonadal hormones (81,120). Ultimately, models are tools we use to answer questions, and the suitability of any model is defined by the question. If the question is "how or why do males and females differ?", an optimal model would expose males and females to the same stress manipulations. If the question is "what are the mechanisms of susceptibility within each sex?", a different, yet equally valid, question, an optimal model could be one adapted specifically to induce stress in females (or males). While there is no single ideal female stress model, several strong models exist, and tools are available to further refine stress paradigms for female rodents. CVS is a standardized model that is easily titrated to test manipulations that induce susceptibility or resilience. Adding an additional stress of a social nature, such as social isolation or social instability, could further enhance this model. Chronic nondiscriminatory social defeat stress, female CFW defeat, and witness defeat are all potentially promising variants of social defeat stress that produce important phenotypic divergence. Finally, social isolation stress is easily employed to probe effects of stress across the life span, particularly important given that depression is often first diagnosed in adolescence and continues to affect women into late adulthood. Establishing rigorous and widely replicable chronic stress models in females is critical to advance research into both the biological basis of sex differences and sex-specific mechanisms of stress susceptibility.

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ARTICLE INFORMATION

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REFERENCES

1. Malhi GS, Mann JJ (2018): Depression. Lancet 392:2299–2312.
2. Whiteford HA, Degenhardt L, Rehm J, Baxter AJ, Ferrari AJ, Erskine HE, *et al.* (2013): Global burden of disease attributable to mental and substance use disorders: Findings from the Global Burden of Disease Study 2010. Lancet 382:1575–1586.
3. Kuehner C (2003): Gender differences in unipolar depression: An update of epidemiological findings and possible explanations. Acta Psychiatr Scand 108:163–174.
4. Seedat S, Scott KM, Angermeyer MC, Berglund P, Bromet EJ, Brugha TS, *et al.* (2009): Cross-national associations between gender and mental disorders in the World Health Organization World Mental Health Surveys. Arch Gen Psychiatry 66:785–795.
5. Marcus SM, Kerber KB, Rush AJ, Wisniewski SR, Nierenberg A, Balasubramani GK, *et al.* (2008): Sex differences in depression symptoms in treatment-seeking adults: Confirmatory analyses from

the Sequenced Treatment Alternatives to Relieve Depression study. Compr Psychiatry 49:238–246.
6. Silverstein B, Edwards T, Gamma A, Ajdacic-Gross V, Rossler W, Angst J (2013): The role played by depression associated with somatic symptomatology in accounting for the gender difference in the prevalence of depression. Soc Psychiatry Psychiatr Epidemiol 48:257–263.
7. Blanco C, Vesga-López O, Stewart JW, Liu SM, Grant BF, Hasin DS (2012): Epidemiology of major depression with atypical features: Results from the National Epidemiologic Survey on Alcohol and Related Conditions (NESARC). J Clin Psychiatry 73:224–232.
8. Khan A, Brodhead AE, Schwartz KA, Kolts RL, Brown WA (2005): Sex differences in antidepressant response in recent antidepressant clinical trials. J Clin Psychopharmacol 25:318–324.
9. Kornstein SG, Schatzberg AF, Thase ME, Yonkers KA, McCullough JP, Keitner GI, *et al.* (2000): Gender differences in treatment response to sertraline versus imipramine in chronic depression. Am J Psychiatry 157:1445–1452.
10. Beery AK, Zucker I (2011): Sex bias in neuroscience and biomedical research. Neurosci Biobehav Rev 35:565–572.
11. Palanza P, Parmigiani S (2017): How does sex matter? Behavior, stress and animal models of neurobehavioral disorders. Neurosci Biobehav Rev 76:134–143.
12. Shansky RM (2019): Are hormones a "female problem" for animal research? Science 364:825–826.
13. Prendergast BJ, Onishi KG, Zucker I (2014): Female mice liberated for inclusion in neuroscience and biomedical research. Neurosci Biobehav Rev 40:1–5.
14. Machida T, Yonezawa Y, Noumura T (1981): Age-associated changes in plasma testosterone levels in male mice and their relation to social dominance or subordinance. Horm Behav 15:238–245.
15. Sayegh JF, Kobor G, Lajtha A, Vadasz C (1990): Effects of social isolation and the time of day on testosterone levels in plasma of C57BL/6By and BALB/cBy mice. Steroids 55:79–82.
16. Lucas LA, Eleftheriou BE (1980): Circadian variation in concentrations of testosterone in the plasma of male mice: A difference between BALB/cBy and C57BL/6By inbred strains. J Endocrinol 87:37–46.
17. Miller LR, Marks C, Becker JB, Hurn PD, Chen WJ, Woodruff T, *et al.* (2017): Considering sex as a biological variable in preclinical research. FASEB J 31:29–34.
18. LeGates TA, Kvarta MD, Thompson SM (2019): Sex differences in antidepressant efficacy. Neuropsychopharmacology 44:140–154.
19. Czéh B, Fuchs E, Wiborg O, Simon M (2016): Animal models of major depression and their clinical implications. Prog Neuropsychopharmacol Biol Psychiatry 64:293–310.
20. Yan HC, Cao X, Das M, Zhu XH, Gao TM (2010): Behavioral animal models of depression. Neurosci Bull 26:327–337.
21. Belzung C, Lemoine M (2011): Criteria of validity for animal models of psychiatric disorders: Focus on anxiety disorders and depression. Biol Mood Anxiety Disord 1:9.
22. Gururajan A, Reif A, Cryan JF, Slattery DA (2019): The future of rodent models in depression research. Nat Rev Neurosci 20:686–701.
23. Dadomo H, Gioiosa L, Cigalotti J, Ceresini G, Parmigiani S, Palanza P (2018): What is stressful for females? Differential effects of unpredictable environmental or social stress in CD1 female mice. Horm Behav 98:22–32.
24. Kokras N, Dalla C (2014): Sex differences in animal models of psychiatric disorders. Br J Pharmacol 171:4595–4619.
25. Zimmerberg B, Farley MJ (1993): Sex differences in anxiety behavior in rats: Role of gonadal hormones. Physiol Behav 54:1119–1124.
26. An XL, Zou JX, Wu RY, Yang Y, Tai FD, Zeng SY, *et al.* (2011): Strain and sex differences in anxiety-like and social behaviors in C57BL/6J and BALB/cJ mice. Exp Anim 60:111–123.
27. Roman E, Arborelius L (2009): Male but not female Wistar rats show increased anxiety-like behaviour in response to bright light in the defensive withdrawal test. Behav Brain Res 202:303–307.
28. Imhof JT, Coelho ZM, Schmitt ML, Morato GS, Carobrez AP (1993): Influence of gender and age on performance of rats in the elevated plus maze apparatus. Behav Brain Res 56:177–180.
29. Rodgers RJ, Cole JC (1993): Influence of social isolation, gender, strain, and prior novelty on plus-maze behaviour in mice. Physiol Behav 54:729–736.
30. Bogdanova OV, Kanekar S, D'Anci KE, Renshaw PF (2013): Factors influencing behavior in the forced swim test. Physiol Behav 118:227–239.
31. Campbell T, Lin S, DeVries C, Lambert K (2003): Coping strategies in male and female rats exposed to multiple stressors. Physiol Behav 78:495–504.
32. Dalla C, Antoniou K, Drossopoulou G, Xagoraris M, Kokras N, Sfikakis A, Papadopoulou-Daifoti Z (2005): Chronic mild stress impact: Are females more vulnerable? Neuroscience 135:703–714.
33. Dalla C, Antoniou K, Kokras N, Drossopoulou G, Papathanasiou G, Bekris S, *et al.* (2008): Sex differences in the effects of two stress paradigms on dopaminergic neurotransmission. Physiol Behav 93:595–605.
34. Konkle AT, Baker SL, Kentner AC, Barbagallo LS, Merali Z, Bielajew C (2003): Evaluation of the effects of chronic mild stressors on hedonic and physiological responses: Sex and strain compared. Brain Res 992:227–238.
35. Mateus-Pinheiro A, Patrício P, Alves ND, Machado-Santos AR, Morais M, Bessa JM, *et al.* (2014): The Sweet Drive Test: Refining phenotypic characterization of anhedonic behavior in rodents. Front Behav Neurosci 8:74.
36. Shansky RM (2015): Sex differences in PTSD resilience and susceptibility: Challenges for animal models of fear learning. Neurobiol Stress 1:60–65.
37. Duque-Wilckens N, Torres LY, Yokoyama S, Minie VA, Tran AM, Petkova SP, *et al.* (2020): Extrahypothalamic oxytocin neurons drive stress-induced social vigilance and avoidance. Proc Natl Acad Sci U S A 117:26406–26413.
38. Newman EL, Covington HE 3rd, Suh J, Bicakci MB, Ressler KJ, DeBold JF, Miczek KA (2019): Fighting females: Neural and behavioral consequences of social defeat stress in female mice. Biol Psychiatry 86:657–668.
39. Antoniuk S, Bijata M, Ponimaskin E, Wlodarczyk J (2019): Chronic unpredictable mild stress for modeling depression in rodents: Meta-analysis of model reliability. Neurosci Biobehav Rev 99:101–116.
40. Matthews K, Forbes N, Reid IC (1995): Sucrose consumption as an hedonic measure following chronic unpredictable mild stress. Physiol Behav 57:241–248.
41. Liu MY, Yin CY, Zhu LJ, Zhu XH, Xu C, Luo CX, *et al.* (2018): Sucrose preference test for measurement of stress-induced anhedonia in mice. Nat Protoc 13:1686–1698.
42. Franceschelli A, Herchick S, Thelen C, Papadopoulou-Daifoti Z, Pitychoutis PM (2014): Sex differences in the chronic mild stress model of depression. Behav Pharmacol 25:372–383.
43. Vollmayr B, Gass P (2013): Learned helplessness: Unique features and translational value of a cognitive depression model. Cell Tissue Res 354:171–178.
44. Maier SF, Seligman MEP (2016): Learned helplessness at fifty: Insights from neuroscience. Psychol Rev 123:349–367.
45. Seligman ME, Beagley G (1975): Learned helplessness in the rat. J Comp Physiol Psychol 88:534–541.
46. Chourbaji S, Zacher C, Sanchis-Segura C, Dormann C, Vollmayr B, Gass P (2005): Learned helplessness: Validity and reliability of depressive-like states in mice. Brain Res Brain Res Protoc 16:70–78.
47. Kott JM, Mooney-Leber SM, Shoubah FA, Brummelte S (2016): Effectiveness of different corticosterone administration methods to elevate corticosterone serum levels, induce depressive-like behavior, and affect neurogenesis levels in female rats. Neuroscience 312:201–214.
48. Kokras N, Krokida S, Varoudaki TZ, Dalla C (2021): Do corticosterone levels predict female depressive-like behavior in rodents? J Neurosci Res 99:324–331.

49. Chiba S, Numakawa T, Ninomiya M, Richards MC, Wakabayashi C, Kunugi H (2012): Chronic restraint stress causes anxiety- and depression-like behaviors, downregulates glucocorticoid receptor expression, and attenuates glutamate release induced by brain-derived neurotrophic factor in the prefrontal cortex. Prog Neuropsychopharmacol Biol Psychiatry 39:112–119.
50. Luo YW, Xu Y, Cao WY, Zhong XL, Duan J, Wang XQ, *et al.* (2015): Insulin-like growth factor 2 mitigates depressive behavior in a rat model of chronic stress. Neuropharmacology 89:318–324.
51. Zain MA, Pandy V, Majeed ABA, Wong WF, Mohamed Z (2019): Chronic restraint stress impairs sociability but not social recognition and spatial memory in C57BL/6J mice. Exp Anim 68:113–124.
52. Zhu S, Shi R, Wang J, Wang JF, Li XM (2014): Unpredictable chronic mild stress not chronic restraint stress induces depressive behaviours in mice. Neuroreport 25:1151–1155.
53. Toth I, Neumann ID (2013): Animal models of social avoidance and social fear. Cell Tissue Res 354:107–118.
54. Willner P (2017): The chronic mild stress (CMS) model of depression: History, evaluation and usage. Neurobiol Stress 6:78–93.
55. Mutlu O, Gumuslu E, Ulak G, Celikyurt IK, Kokturk S, Kır HM, *et al.* (2012): Effects of fluoxetine, tianeptine and olanzapine on unpredictable chronic mild stress-induced depression-like behavior in mice. Life Sci 91:1252–1262.
56. Willner P, Towell A, Sampson D, Sophokleous S, Muscat R (1987): Reduction of sucrose preference by chronic unpredictable mild stress, and its restoration by a tricyclic antidepressant. Psychopharmacology (Berl) 93:358–364.
57. Willner P (2005): Chronic mild stress (CMS) revisited: Consistency and behavioural-neurobiological concordance in the effects of CMS. Neuropsychobiology 52:90–110.
58. Abelaira HM, Réus GZ, Quevedo J (2013): Animal models as tools to study the pathophysiology of depression. Braz J Psychiatry 35(suppl 2):S112–S120.
59. Ma L, Xu Y, Wang G, Li R (2019): What do we know about sex differences in depression: A review of animal models and potential mechanisms. Prog Neuropsychopharmacol Biol Psychiatry 89:48–56.
60. Lu Y, Ho CS, Liu X, Chua AN, Wang W, McIntyre RS, Ho RC (2017): Chronic administration of fluoxetine and pro-inflammatory cytokine change in a rat model of depression. PLoS One 12:e0186700.
61. Filho CB, Jesse CR, Donato F, Giacomeli R, Del Fabbro L, da Silva Antunes M, *et al.* (2015): Chronic unpredictable mild stress decreases BDNF and NGF levels and Na(+),K(+)-ATPase activity in the hippocampus and prefrontal cortex of mice: Antidepressant effect of chrysin. Neuroscience 289:367–380.
62. Borrow AP, Bales NJ, Stover SA, Handa RJ (2018): Chronic variable stress induces sex-specific alterations in social behavior and neuropeptide expression in the mouse. Endocrinology 159:2803–2814.
63. Liu J, Dietz K, Hodes GE, Russo SJ, Casaccia P (2018): Widespread transcriptional alternations in oligodendrocytes in the adult mouse brain following chronic stress. Dev Neurobiol 78:152–162.
64. Johnson A, Rainville JR, Rivero-Ballon GN, Dhimitri K, Hodes GE (2021): Testing the limits of sex differences using variable stress. Neuroscience 454:72–84.
65. Labonté B, Engmann O, Purushothaman I, Menard C, Wang J, Tan C, *et al.* (2017): Sex-specific transcriptional signatures in human depression. Nat Med 23:1102–1111.
66. LaPlant Q, Chakravarty S, Vialou V, Mukherjee S, Koo JW, Kalahasti G, *et al.* (2009): Role of nuclear factor kappaB in Ovarian Hormone-Mediated Stress Hypersensitivity in Female Mice. Biol Psychiatry 65:874–880.
67. Hodes GE, Pfau ML, Purushothaman I, Ahn HF, Golden SA, Christoffel DJ, *et al.* (2015): Sex differences in nucleus accumbens transcriptome profiles associated with susceptibility versus resilience to subchronic variable stress. J Neurosci 35:16362–16376.
68. Pfau ML, Purushothaman I, Feng J, Golden SA, Aleyasin H, Lorsch ZS, *et al.* (2016): Integrative analysis of sex-specific microRNA networks following stress in mouse nucleus accumbens. Front Mol Neurosci 9:144.
69. Brancato A, Bregman D, Ahn HF, Pfau ML, Menard C, Cannizzaro C, *et al.* (2017): Sub-chronic variable stress induces sex-specific effects on glutamatergic synapses in the nucleus accumbens. Neuroscience 350:180–189.
70. Zhang S, Zhang H, Ku SM, Juarez B, Morel C, Tzavaras N, *et al.* (2018): Sex differences in the neuroadaptations of reward-related circuits in response to subchronic variable stress. Neuroscience 376:108–116.
71. Williams ES, Manning CE, Eagle AL, Swift-Gallant A, Duque-Wilckens N, Chinnusamy S, *et al.* (2020): Androgen-dependent excitability of mouse ventral hippocampal afferents to nucleus accumbens underlies sex-specific susceptibility to stress. Biol Psychiatry 87:492–501.
72. Muir J, Tse YC, Iyer ES, Biris J, Cvetkovska V, Lopez J, Bagot RC (2020): Ventral hippocampal afferents to nucleus accumbens encode both latent vulnerability and stress-induced susceptibility. Biol Psychiatry 88:843–854.
73. Kuehner C (2017): Why is depression more common among women than among men? Lancet Psychiatry 4:146–158.
74. Zaletel I, Filipović D, Puškaš N (2017): Hippocampal BDNF in physiological conditions and social isolation. Rev Neurosci 28:675–692.
75. Palanza P, Gioiosa L, Parmigiani S (2001): Social stress in mice: Gender differences and effects of estrous cycle and social dominance. Physiol Behav 73:411–420.
76. Singewald GM, Nguyen NK, Neumann ID, Singewald N, Reber SO (2009): Effect of chronic psychosocial stress-induced by subordinate colony (CSC) housing on brain neuronal activity patterns in mice. Stress 12:58–69.
77. Wallace DL, Han MH, Graham DL, Green TA, Vialou V, Iñiguez SD, *et al.* (2009): CREB regulation of nucleus accumbens excitability mediates social isolation-induced behavioral deficits. Nat Neurosci 12:200–209.
78. Grippo AJ, Cushing BS, Carter CS (2007): Depression-like behavior and stressor-induced neuroendocrine activation in female prairie voles exposed to chronic social isolation. Psychosom Med 69:149–157.
79. Martin AL, Brown RE (2010): The lonely mouse: Verification of a separation-induced model of depression in female mice. Behav Brain Res 207:196–207.
80. Liu N, Wang Y, An AY, Banker C, Qian YH, O'Donnell JM (2020): Single housing-induced effects on cognitive impairment and depression-like behavior in male and female mice involve neuroplasticity-related signaling. Eur J Neurosci 52:2694–2704.
81. Sarkar A, Kabbaj M (2016): Sex differences in effects of ketamine on behavior, spine density, and synaptic proteins in socially isolated rats. Biol Psychiatry 80:448–456.
82. Herzog CJ, Czéh B, Corbach S, Wuttke W, Schulte-Herbrüggen O, Hellweg R, *et al.* (2009): Chronic social instability stress in female rats: A potential animal model for female depression. Neuroscience 159:982–992.
83. Yohn CN, Ashamalla SA, Bokka L, Gergues MM, Garino A, Samuels BA (2019): Social instability is an effective chronic stress paradigm for both male and female mice. Neuropharmacology 160:107780.
84. Goñi-Balentziaga O, Perez-Tejada J, Renteria-Dominguez A, Lebeña A, Labaka A (2018): Social instability in female rodents as a model of stress related disorders: A systematic review. Physiol Behav 196:190–199.
85. Haller J, Fuchs E, Halász J, Makara GB (1999): Defeat is a major stressor in males while social instability is stressful mainly in females: Towards the development of a social stress model in female rats. Brain Res Bull 50:33–39.
86. Sterlemann V, Ganea K, Liebl C, Harbich D, Alam S, Holsboer F, *et al.* (2008): Long-term behavioral and neuroendocrine alterations following chronic social stress in mice: Implications for stress-related disorders. Horm Behav 53:386–394.
87. Schmidt MV, Scharf SH, Liebl C, Harbich D, Mayer B, Holsboer F, Müller MB (2010): A novel chronic social stress paradigm in female mice. Horm Behav 57:415–420.

88. Baranyi J, Bakos N, Haller J (2005): Social instability in female rats: The relationship between stress-related and anxiety-like consequences. Physiol Behav 84:511–518.
89. Haller J, Baranyi J, Bakos N, Halász J (2004): Social instability in female rats: Effects on anxiety and buspirone efficacy. Psychopharmacology (Berl) 174:197–202.
90. Nowacka-Chmielewska MM, Kasprowska-Liśkiewicz D, Barski JJ, Obuchowicz E, Małecki A (2017): The behavioral and molecular evaluation of effects of social instability stress as a model of stress-related disorders in adult female rats. Stress 20:549–561.
91. Planchez B, Surget A, Belzung C (2019): Animal models of major depression: Drawbacks and challenges. J Neural Transm (Vienna) 126:1383–1408.
92. Krishnan V, Han MH, Graham DL, Berton O, Renthal W, Russo SJ, *et al.* (2007): Molecular adaptations underlying susceptibility and resistance to social defeat in brain reward regions. Cell 131:391–404.
93. Hollis F, Kabbaj M (2014): Social defeat as an animal model for depression. ILAR J 55:221–232.
94. Golden SA, Covington HE 3rd, Berton O, Russo SJ (2011): A standardized protocol for repeated social defeat stress in mice. Nat Protoc 6:1183–1191.
95. Berton O, McClung CA, Dileone RJ, Krishnan V, Renthal W, Russo SJ, *et al.* (2006): Essential role of BDNF in the mesolimbic dopamine pathway in social defeat stress. Science 311:864–868.
96. Donahue RJ, Muschamp JW, Russo SJ, Nestler EJ, Carlezon WA Jr (2014): Effects of striatal ΔFosB overexpression and ketamine on social defeat stress-induced anhedonia in mice. Biol Psychiatry 76:550–558.
97. Vialou V, Robison AJ, Laplant QC, Covington HE 3rd, Dietz DM, Ohnishi YN, *et al.* (2010): DeltaFosB in brain reward circuits mediates resilience to stress and antidepressant responses. Nat Neurosci 13:745–752.
98. Harris AZ, Atsak P, Bretton ZH, Holt ES, Alam R, Morton MP, *et al.* (2018): A novel method for chronic social defeat stress in female mice. Neuropsychopharmacology 43:1276–1283.
99. Takahashi A, Chung JR, Zhang S, Zhang H, Grossman Y, Aleyasin H, *et al.* (2017): Establishment of a repeated social defeat stress model in female mice. Sci Rep 7:12838.
100. Issler O, van der Zee YY, Ramakrishnan A, Wang J, Tan C, Loh YE, *et al.* (2020): Sex-specific role for the long non-coding RNA LINC00473 in depression. Neuron 106:912–926.e5.
101. Yohn CN, Dieterich A, Bazer AS, Maita I, Giedraitis M, Samuels BA (2019): Chronic non-discriminatory social defeat is an effective chronic stress paradigm for both male and female mice. Neuropsychopharmacology 44:2220–2229.
102. Solomon MB (2017): Evaluating social defeat as a model for psychopathology in adult female rodents. J Neurosci Res 95:763–776.
103. Bourke CH, Neigh GN (2012): Exposure to repeated maternal aggression induces depressive-like behavior and increases startle in adult female rats. Behav Brain Res 227:270–275.
104. Shimamoto A, Debold JF, Holly EN, Miczek KA (2011): Blunted accumbal dopamine response to cocaine following chronic social stress in female rats: Exploring a link between depression and drug abuse. Psychopharmacol (Berl) 218:271–279.
105. Jacobson-Pick S, Audet MC, McQuaid RJ, Kalvapalle R, Anisman H (2013): Social agonistic distress in male and female mice: Changes of behavior and brain monoamine functioning in relation to acute and chronic challenges. PLoS One 8:e60133.
106. Trainor BC, Pride MC, Villalon Landeros R, Knoblauch NW, Takahashi EY, Silva AL, Crean KK (2011): Sex differences in social interaction behavior following social defeat stress in the monogamous California mouse (Peromyscus californicus). PLoS One 6: e17405.
107. Kingston RC, Smith M, Lacey T, Edwards M, Best JN, Markham CM (2018): Voluntary exercise increases resilience to social defeat stress in Syrian hamsters. Physiol Behav 188:194–198.
108. Steinman MQ, Trainor BC (2017): Sex differences in the effects of social defeat on brain and behavior in the California mouse: Insights from a monogamous rodent. Semin Cell Dev Biol 61:92–98.
109. Greenberg GD, Laman-Maharg A, Campi KL, Voigt H, Orr VN, Schaal L, Trainor BC (2014): Sex differences in stress-induced social withdrawal: Role of brain derived neurotrophic factor in the bed nucleus of the stria terminalis. Front Behav Neurosci 7:223.
110. Logan RW (2019): Adapting social defeat stress for female mice using species-typical interfemale aggression. Biol Psychiatry 86:e31–e32.
111. Sial OK, Warren BL, Alcantara LF, Parise EM, Bolaños-Guzmán CA (2016): Vicarious social defeat stress: Bridging the gap between physical and emotional stress. J Neurosci Methods 258:94–103.
112. Warren BL, Vialou VF, Iñiguez SD, Alcantara LF, Wright KN, Feng J, *et al.* (2013): Neurobiological sequelae of witnessing stressful events in adult mice. Biol Psychiatry 73:7–14.
113. Patki G, Salvi A, Liu H, Salim S (2015): Witnessing traumatic events and post-traumatic stress disorder: Insights from an animal model. Neurosci Lett 600:28–32.
114. Nakatake Y, Furuie H, Yamada M, Kuniishi H, Ukezono M, Yoshizawa K, Yamada M (2020): The effects of emotional stress are not identical to those of physical stress in mouse model of social defeat stress. Neurosci Res 158:56–63.
115. Patki G, Solanki N, Salim S (2014): Witnessing traumatic events causes severe behavioral impairments in rats. Int *J* Neuropsychopharmacol 17:2017–2029.
116. Iñiguez SD, Flores-Ramirez FJ, Riggs LM, Alipio JB, Garcia-Carachure I, Hernandez MA, *et al.* (2018): Vicarious social defeat stress induces depression-related outcomes in female mice. Biol Psychiatry 83:9–17.
117. Finnell JE, Muniz BL, Padi AR, Lombard CM, Moffitt CM, Wood CS, *et al.* (2018): Essential role of ovarian hormones in susceptibility to the consequences of witnessing social defeat in female rats. Biol Psychiatry 84:372–382.
118. Gaskill BN, Karas AZ, Garner JP, Pritchett-Corning KR (2013): Nest building as an indicator of health and welfare in laboratory mice. J Vis Exp 82:51012.
119. Neely CLC, Pedemonte KA, Boggs KN, Flinn JM (2019): Nest building behavior as an early indicator of behavioral deficits in mice. J Vis Exp 152.
120. Carrier N, Kabbaj M (2013): Sex differences in the antidepressant-like effects of ketamine. Neuropharmacology 70:27–34.
121. Dalla C, Edgecomb C, Whetstone AS, Shors TJ (2008): Females do not express learned helplessness like males do. Neuropsychopharmacology 33:1559–1569.
122. Caldarone BJ, George TP, Zachariou V, Picciotto MR (2000): Gender differences in learned helplessness behavior are influenced by genetic background. Pharmacol Biochem Behav 66:811–817.
123. Ngoupaye GT, Yassi FB, Bahane DAN, Bum EN (2018): Combined corticosterone treatment and chronic restraint stress lead to depression associated with early cognitive deficits in mice. Metab Brain Dis 33:421–431.